Neogenin and repulsive guidance molecule signaling in the central nervous system

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The repulsive guidance molecule (RGM) is a membrane-bound protein that was originally identified as an axon guidance molecule in the visual system. Functional studies have revealed that it has roles in axon guidance and laminar patterning in *Xenopus* and chick embryos, and in controlling cephalic neural tube closure in mouse embryos. The recent identification of neogenin as a receptor for RGM has provided evidence of the diverse functions of this ligand–receptor pair. Re-expression of RGM is observed after injury in the adult human and rat central nervous systems. Inhibition of RGM enhances growth of injured axons and promotes functional recovery after spinal cord injury in rats. Thus, re-expression of embryonic repulsive cues in adult tissues contributes to failure of axon regeneration in the central nervous system.

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Introduction

Repulsive guidance molecule (RGM) was originally identified as a membrane-bound protein with repulsive properties and the ability to induce collapse of the growth cone in the chick retinotectal system [1]. The molecule was subsequently isolated and cloned from the chick tectum [2]. Chick RGM mRNA showed a graded pattern of expression in the tectum, with higher expression in the posterior tectum and lower expression in the anterior tectum. It was found to repel temporal retinal axons in the stripe assay and to collapse their growth cones; thus, it was thought that it might be involved in topographic map formation [2]. Since the time of these observations, however, our knowledge regarding RGM has increased and unexpected diverse functions of RGMs have been uncovered [3,4].

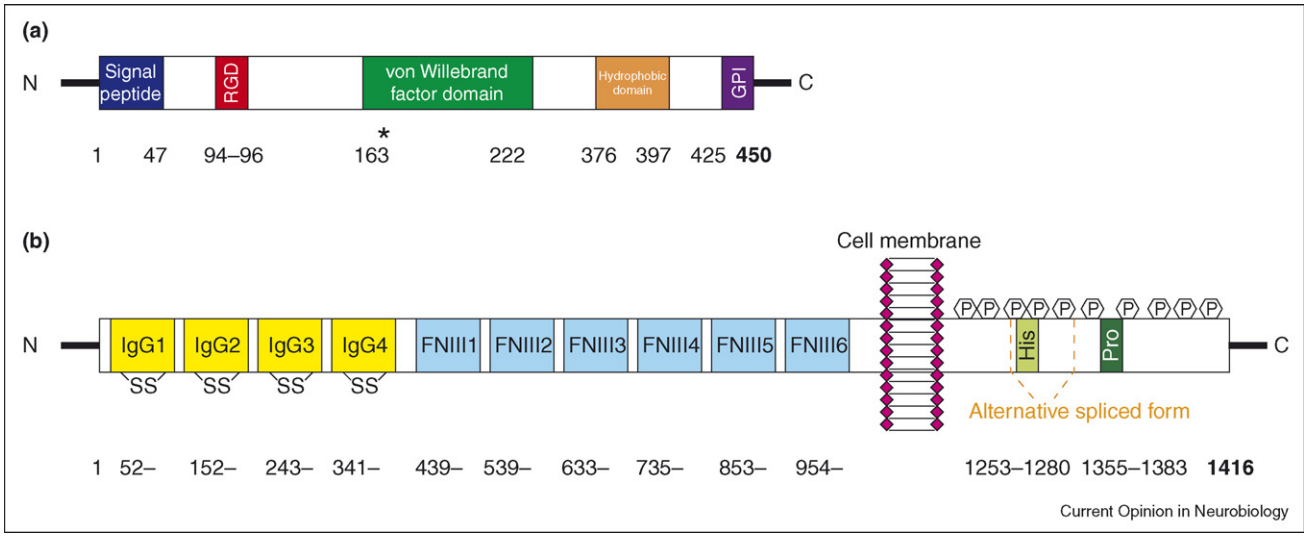
RGM is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein that does not share significant homology with any other known protein (Figure 1). It contains a putative autoproteolytic or unstable cleavage site; an N-terminal signal peptide; an Arg-Gly-Asp (RGD) site; a partial, structurally related, von Willebrand factor type D domain; and a hydrophobic domain of unknown function [2,4]. Vertebrates have three homologs of RGM, namely, RGMa, RGMb (also known as DRAGON) and RGMc (also known as hemojuvelin or HJV), of which RGMa is the most closely related ortholog of chick RGM. This review particularly focuses on critical roles of RGM in axon guidance during the developmental stage and in inhibition of axon growth after injury in the adult central nervous system (CNS).

Axon navigation by RGM during development

As mentioned above, RGM in the chick tectum shows a graded pattern of expression. By contrast, this pattern of RGMa expression is not seen in the mouse superior colliculus and RGMa mutant mice show normal projection patterns of retinal axons in the superior colliculus [5]. The reason for the phenotypic differences between chick and mice remains unknown, because compensatory upregulation of gene expression of other RGMs or ephrin family members has not been observed in mice [5]. Notably, however, developing retinal ganglion cells express RGMb at high levels and RGMb is also present in the embryonic superior colliculus [5], suggesting that it contributes to the development of retinocollicular projections. *In vivo* studies in RGMb mutant mice are required to determine whether RGM proteins regulate topographic map formation in vertebrates.

Initial evidence showing that RGMa has an axon guidance function *in vivo* has been recently obtained in chick and *Xenopus* embryos [6,7••]. Overexpression or downregulation of RGMa in the chick tectum was found to result in pathfinding and mapping errors, proving that overexpression or downregulation of RGMa has a negative impact on topographic mapping along the anterior–posterior axis of the chick tectum [6]. In addition, in chick embryos overexpressing RGMa, a laminar erroneous termination of retinal axons was observed and the termination zones were located in deeper tectal layers than in the controls [6]. In *Xenopus* embryos, downregulation of RGMa, netrin-1 or neogenin by a morpholino antisense strategy leads to aberrant projections from the supraoptic tract (SOT) in the forebrain. This aberrant projection phenotype of the RGMa knockdown is very similar in extent and morphology to that of the neogenin knockdown [7••]. Combined partial knockdowns of neogenin

Figure 1



Structure of RGMa and neogenin. The different domains of **(a)** human RGMa and **(b)** its receptor neogenin are shown. RGMa contains a potential cleavage site at N-terminal amino acid 168 (indicated by the asterisk). Potential phosphorylation sites are marked (hexagonal boxes).

and RGMa, or of neogenin and netrin-1, result in a significant increase in the number of aberrant SOT projections, thereby suggesting that neogenin, RGMa and netrin-1 are involved in the same signaling pathway during SOT development [7••].

Another study has shown that RGM functions as an axon guidance molecule in the developing mouse hippocampus [8]. RGMa is expressed in the inner molecular layer of the dentate gyrus, whereas fibers from the entorhinal cortex terminate in the outer molecular layers and are repelled by RGMa. Addition of a function-blocking RGMa antibody in an organotypic entorhinal cortex–hippocampus co-culture system results in aberrant projections of entorhinal fibers and abolition of their layer-specific termination pattern [8]. Thus, RGMa seems to restrict entorhinal fibers to their correct layer — namely, the outer molecular layer. This function is reminiscent of the aberrant layering of retinal fibers in the tectum of chick embryos overexpressing RGMa.

RGM is necessary for neural tube closure

RGMa and RGMb show abundant expression in early stages of the development of the mouse CNS. Expression of RGMa and RGMb is first noted at the tips of the neural fold at embryonic day 8.5 (E8.5) to E9.5, precisely at the beginning of neural tube closure in mice [5]. Strong and mostly non-overlapping expression domains of mouse RGMa and RGMb have been observed in the embryonic brain and these expression domains have been found to persist in several brain regions after birth [5,9–11].

Consistent with these expression patterns, an unexpected role of RGMa in early embryonic development was identified from the analysis of RGMa knockout mice. These mice show deficits in neural tube closure at the cephalic level, resulting in an exencephalic phenotype with major morphological defects of the dorsal brain structures [5]. A neural tube defect has also been reported in zebrafish after knockdown of the *Neogenin* gene, even though the mode of neural tube formation differs from that of mice [12].

Neogenin as a receptor for RGM

The receptor for RGM has been identified as neogenin [13], which was originally isolated from embryonic chicken cerebellum as a homolog of the netrin receptor 'deleted in colorectal cancer' (DCC) [14]. The extracellular domain of human neogenin contains four V-shaped immunoglobulin-like domains and six fibronectin type III (FNIII)-like domains (Figure 1). Netrin-1 also binds to neogenin, but the binding affinity of RGM for neogenin (K_d = 230 pM) is much higher than that of netrin-1 (K_d = 2 nM). Both RGM and netrin-1 bind to the FNIII-like domain of neogenin [13,15]. It is not known whether netrin-1 modulates the binding of RGM to neogenin or the subsequent signaling pathway; such information would be useful for assessing the possibility that fine-tuning in axon guidance is regulated by the competition of these molecules for the same or overlapping binding sites.

Neogenin and DCC belong to the structurally diverse family of so-called 'dependence receptors' — a denomination to indicate that the absence of the ligand induces self-activation of the receptor and its subsequent proteolytic processing, thereby triggering apoptotic cell death [16,17]. This assumption is based on the finding that

overexpression of neogenin or downregulation of RGMa by short interfering RNA at E1.5 in the area of the developing dorsal metencephalon, mesencephalon and caudal diencephalons in chick embryos results in an increase in the number of cells positive for TdT-mediated dUTP nick end labeling [18]. Apoptosis is caused by the proteolytic cleavage of the receptor by activated caspase-3, although knowledge regarding the underlying signal transduction mechanisms is limited.

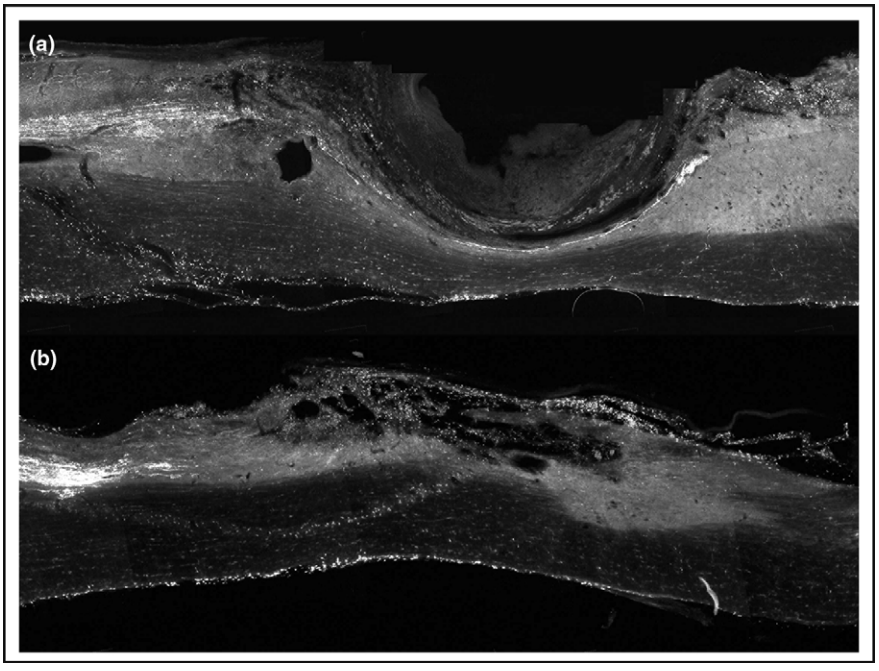
A subsequent study has suggested that RGMa might have an active role in chick embryo development, in addition to rescuing cells by suppressing neogenin-mediated cell death. Overexpression of RGMa in the neural tube promotes neuronal differentiation, whereas suppression of RGMa represses this process [6]. Thus, RGMa not only suppresses neogenin-induced cell death but also enhances neuronal differentiation. Final cell fate decisions might be made on the basis of the relative expression levels of RGMa and neogenin. No alterations in cellular proliferation or neuronal differentiation have been reported in the RGMa mutant mice [5], however, suggesting that redundant mechanisms exist in rodents.

RGMa inhibits axon regeneration and functional recovery in the injured CNS

In the adult mammalian CNS, injured axons show very limited regenerative ability. Owing to a lack of appropriate axonal regeneration, traumatic damage to the adult brain and spinal cord frequently causes permanent neuronal deficits. Several myelin-associated proteins in the CNS have been identified as inhibitors of axonal regeneration after injury of the adult vertebrate CNS. Among these inhibitors, myelin-associated glycoprotein (MAG), Nogo and oligodendrocyte-myelin glycoprotein have been well characterized [19]. A recent report now suggests that RGMa also functions as a myelin-derived neurite outgrowth inhibitor *in vitro* and *in vivo* [20••].

Under pathological conditions, RGMa is upregulated around the lesion site in rats with spinal cord injury (SCI) [21]. Neogenin is expressed widely in the adult CNS and spinal cord, and is distributed predominantly in the gray matter [22]. To assess whether RGMa is involved in the inhibition of axon regeneration, a function-blocking antibody was used to treat adult rats with SCI [20••]. The RGMa antibody was locally administered through an osmotic minipump for two weeks after injury. Ten weeks after SCI, tracing of the corticospinal tract (CST) revealed massive growth and sprouting of the axons beyond the lesion epicenter in rats treated with the RGMa antibody, whereas slight growth of CST fibers was observed in those treated with the control antibody (Figure 2) [20••]. The extensive regrowth or sprouting of fibers in rats treated with the RGMa antibody correlated well with the improved functional recovery in these animals. Thus,

Figure 2



The anti-RGMa antibody promotes the regeneration of CST axons after SCI. Representative images of CST fibers, anterograde labeled with biotinylated dextranamine, in rat spinal cord 10 weeks after injury: **(a)** rats treated with anti-RGMa antibody; **(b)** rats treated with control IgG. Rostral is to the left. Regeneration or sprouting of injured axons is massively induced by treatment with anti-RGMa antibody. Figure reproduced, with permission, from [20••].

RGMa has been shown to be a valid example of an axon growth inhibitor in adult rats. In agreement with these observations, immediate (one day) and long-lasting (weeks or months) upregulation of RGM expression has been observed at the lesion site and in the scar tissue of humans suffering from focal cerebral ischemia or a traumatic brain injury [23].

Because RGMa is expressed in CNS myelin purified from an adult rat brain and the function-blocking RGMa antibody decreased the inhibitory effect of myelin on neurite growth *in vitro* [20••], RGMa must also be a myelin-derived inhibitor of axon growth. After SCI, however, induction of RGMa in the epicenter area was also found in IB-4-positive microglia and/or macrophages [20••] — a cell type that also expresses netrin-1, the other ligand of neogenin, in spinally injured mice [24]. In adult rats, netrin-1 is present in the spinal cord in both the grey and white matter [22], and is upregulated at the lesion site [24]. Because netrin-1 stimulates neurite growth through neogenin [25], preventing the binding of RGMa to neogenin might allow the axons to be navigated by netrin-1.

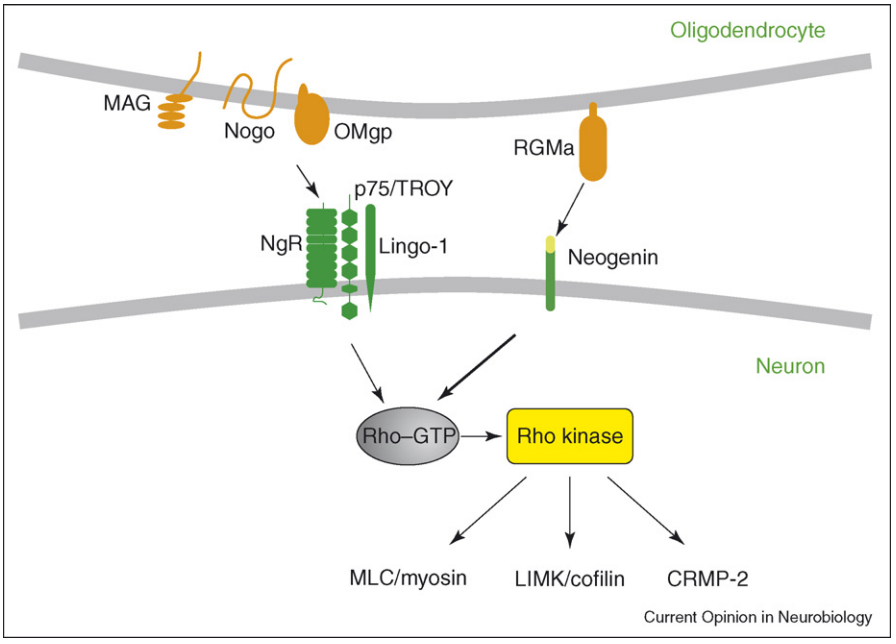
RGM signal transduction during axon inhibition

It is well established that Rho GTPases have key roles in axon guidance and neurite growth [26–28]. Activation of RhoA, in particular, is important both for growth cone collapse and for neurite growth inhibition mediated by the action of several repulsive or inhibitory proteins [29–31], such as ephrin-A5, semaphorin-3A, Wnts, MAG and Nogo-A. Consistent with this notion, RGMa activates RhoA in cerebellar neurons (Figure 3) [20••]. Because inhibition of Rho kinase, a downstream effector of RhoA, prevents the effects of RGMa, activation of RhoA and Rho kinase must be necessary for downstream RGMa signaling.

The mechanism underlying the activation of the RhoA/Rho kinase pathway after RGMa binds to neogenin remains unknown. Rho GTPases are activated by enzymes that enhance GTP binding and activity, such as guanine nucleotide exchange factors (GEFs). Some RhoGEFs might associate with neogenin to induce the activation of RhoA specifically in response to RGM. This possibility is suggested by the following observations: both neogenin and DCC bind to focal adhesion kinase (FAK) through their highly homologous intracellular domain and FAK is associated with the netrin-1-stimulated outgrowth of cortical axons [32,33]. Considering the fact that some RhoGEFs, such as PDZ-RhoGEF and LARG, are phosphorylated by FAK [34,35], these proteins might be involved in RGM signal transduction.

The signal transduction mechanisms underlying other aspects of the RGM–neogenin interaction are mostly unknown. Many signals might be activated constitutively or in a ligand-dependent manner, and could account for

Figure 3



Convergent signaling mediates inhibition of axon growth. RGMa activates the Rho/Rho kinase pathway in neurons through neogenin. Myelin-derived neurite outgrowth inhibitors — namely MAG, Nogo and oligodendrocyte myelin glycoprotein (OMgp) — in oligodendrocytes also induce inhibitory signaling in neurons by activating the Rho/Rho kinase pathway through the Nogo receptor complex comprising p75/Troy, NgR and Lingo-1. Downstream molecules, such as myosin light chain (MLC), LIM kinase (LIMK) and CRMP-2, might have roles in axon growth inhibition.

the various functions of RGM and neogenin. Future studies will address this important issue and will hopefully bridge the significant gap in our knowledge of these signaling mechanisms.

Other aspects of RGM

The diversity of RGM functions might be explained by a recent finding that suggests that members of the bone morphogenetic protein (BMP) family are binding partners of RGMs [36,37•–39•]. All RGMs have been found to enhance signaling of BMP2 and BMP4 *in vitro*. Binding of RGMs to BMPs might place another obstacle in the way of successful neuroregeneration of the injured CNS. Notably, expression of BMP2, BMP6 and BMP7 is induced after injury or insult [40–43].

It will be interesting to assess whether BMPs themselves are axon growth inhibitors in the CNS because BMPs activate LIM kinase — a downstream effector of Rho kinase — directly through the BMP type II receptor [44]. In addition, BMP2 and BMP4 repress oligodendrocyte development by inducing a shift in the commitment of oligodendrocyte progenitors towards the astrocytic lineage. Demyelination is commonly observed after SCI and rapid remyelination might contribute to functional recovery. This process could be prevented by BMPs and upregulation of RGM might potentiate the effect of BMPs.

Conclusions

RGM and neogenin transduce both ligand-independent constitutive and ligand-dependent signals. They have significant roles in axon guidance, cell death, differentiation of neurons and neural tube closure during CNS development. It will be interesting to explore the possible cross-talk among these functions. Do neurons whose axons are inappropriately navigated die in the absence of RGM? What is the key signal that determines cell death or survival?

The functions of RGM and neogenin are, at least in part, associated with the observations that RGM proteins enhance BMP signaling, that netrin-1 binds to neogenin, and that several members of the RGM family exist in mammals. Another interesting aspect of the function of RGM in the adult CNS is that RGM might act as an inhibitory molecule rather than a repulsive molecule. Inhibition of both the RGM–neogenin signal and the BMP signal by a function-blocking antibody might contribute to axon growth and functional recovery after SCI. In this case, LIM kinase might represent another molecular target to develop a fruitful approach towards stimulating the regeneration of nerve fibers. Because RGM has been shown to have diverse functions in the developing nervous system, it might also have roles in addition to inhibiting axon growth, such as regulating cell death and differentiation in the injured adult CNS. Future studies on this topic are required to elucidate the molecular mechanisms of these interesting molecules.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Stahl B, Müller B, Boxberg YV, Cox EC, Bonhoeffer F: **Biochemical characterization of a putative axonal guidance molecule of the chick visual system**. *Neuron* 1990, **5**:735-743.

2. Monnier PP, Sierra A, Macchi P, Deitinghoff L, Andersen JS, Mann M, Flad M, Hornberger M, Stahl B, Bonhoeffer F, Mueller BK: **RGM is a repulsive guidance molecule for retinal axons**. *Nature* 2002, **419**:392-395.

3. Matsunaga E, Chedotal A: **Repulsive guidance molecule/ Neogenin: a novel ligand–receptor system playing multiple roles in neural development**. *Dev Growth Differ* 2004, **46**:481-486.

4. Mueller BK, Yamashita T, Schaffer G, Mueller R: **The role of repulsive guidance molecules (RGMs) in the embryonic and adult vertebrate central nervous system**. *Philos Trans R Soc London B Biol Sci* 2006, **361**:1513-1529.

5. Niederkofler V, Salie R, Sigrist M, Arber S: **Repulsive guidance molecule (RGM) gene function is required for neural tube closure but not retinal topography in the mouse visual system**. *J Neurosci* 2004, **24**:808-818.

6. Matsunaga E, Nakamura H, Chedotal A: **Repulsive guidance molecule plays multiple roles in neuronal differentiation and axon guidance**. *J Neurosci* 2006, **26**:6082-6088.

7. •• Wilson NH, Key B: **Neogenin interacts with RGMa and Netrin-1 to guide axons within the embryonic vertebrate forebrain**. *Dev Biol* 2006, **296**:485-498.
In this detailed study, the authors show for the first time that RGMa and netrin-1 function through neogenin in axon guidance in *Xenopus* forebrains. This paper presents the first *in vivo* data confirming that neogenin-expressing axons respond to proper guidance from both RGMa and netrin-1.

8. Brinks H, Conrad S, Vogt J, Oldekamp J, Sierra A, Deitinghoff L, Bechmann I, Alvarez-Bolado G, Heimrich B, Monnier PP *et al.*: **The repulsive guidance molecule RGMa is involved in the formation of afferent connections in the dentate gyrus**. *J Neurosci* 2004, **24**:3862-3869.

9. Schmidtmer J, Engelkamp D: **Isolation and expression pattern of three mouse homologues of chick RGM**. *Gene Expr Patterns* 2004, **4**:105-110.

10. Oldekamp J, Krämer N, Alvarez-Bolado G, Skutella T: **Expression pattern of the repulsive guidance molecules RGM A, B, and C during mouse development**. *Gene Expr Patterns* 2004, **4**:283-288.

11. Samad TA, Srinivasan A, Karchewski LA, Jeong S-J, Campagna JA, Ji R-R, Fabrizio DA, Zhang Y, Lin HY, Bell E, Woolf CJ: **DRAGON: a member of the repulsive guidance molecule-related family of neuronal- and muscle-expressed membrane proteins is regulated by DRG11 and has neuronal adhesive properties**. *J Neurosci* 2004, **24**:2027-2036.

12. Mawdsley DJ, Cooper HM, Hogan BM, Cody SH, Lieschke GJ, Heath JK: **The Netrin receptor neogenin is required for neural tube formation and somitogenesis in zebrafish**. *Dev Biol* 2004, **269**:302-315.

13. Rajagopalan S, Deitinghoff L, Davis D, Conrad S, Skutella T, Chedotal A, Mueller BK, Strittmatter SM: **Neogenin mediates**

the action of repulsive guidance molecule. *Nat Cell Biol* 2004, **6**:756-762.

14. Vielmetter J, Kayyem JF, Roman JM, Dreyer WJ: **Neogenin, an avian cell surface protein expressed during terminal neuronal differentiation, is closely related to the human tumor suppressor molecule deleted in colorectal cancer**. *J Cell Biol* 1994, **127**:2009-2020.

15. Geisbrecht BV, Dowd KA, Barfield RW, Longo PA, Leahy DJ: **Netrin binds discrete subdomains of DCC and UNC5 and mediates interactions between DCC and heparin**. *J Biol Chem* 2003, **278**:32561-32568.

16. Bredesen DE, Mehlen P, Rabizadeh S: **Apoptosis and dependence receptors: a molecular basis for cellular addiction**. *Physiol Rev* 2004, **84**:411-430.

17. Mehlen P, Fearon ER: **Role of the dependence receptor DCC in colorectal cancer pathogenesis**. *J Clin Oncol* 2004, **22**:3420-3428.

18. Matsunaga E, Tauszig-Delamasure S, Monnier PP, Mueller BK, Strittmatter SM, Mehlen P, Chédotal A: **RGM and its receptor neogenin regulate neuronal survival**. *Nat Cell Biol* 2004, **6**:749-755.

19. Yamashita T, Fujitani M, Yamagishi S, Hata K, Mimura F: **Multiple signals regulate axon regeneration through the nogo receptor complex**. *Mol Neurobiol* 2005, **32**:105-112.

20. •• Hata K, Fujitani M, Yasuda Y, Doya H, Saito T, Yamagishi S, Mueller BK, Yamashita T: **RGMa inhibition promotes axonal growth and recovery after spinal cord injury**. *J Cell Biol* 2006, **173**:47-58.

This is the first report to demonstrate that RGMa is an axon growth inhibitor in adult mammals. The authors use a function-blocking antibody to inhibit the RGMa signal. Rats that undergo spinal cord hemisection show better locomotor recovery and axon growth of the CST after treatment with the function-blocking antibody than after treatment with the control antibody.

21. Schwab JM, Conrad S, Monnier PP, Julien S, Mueller BK, Schluesener HJ: **Spinal cord injury induces lesional expression pattern of the repulsive guidance molecule (RGM)**. *Eur J Neurosci* 2005, **21**:1569-1576.

22. Manitt C, Thompson KM, Kennedy TE: **Developmental shift in expression of Netrin receptors in the rat spinal cord: predominance of UNC-5 homologues in adulthood**. *J Neurosci Res* 2004, **77**:690-700.

23. Schwab JM, Monnier PP, Schluesener HJ, Conrad S, Beschorner R, Chen L, Meyermann R, Mueller BK: **CNS injury-induced RGM (repulsive guidance molecule) expression in the adult human brain**. *Arch Neurol* 2005, **62**:1561-1568.

24. Wehrle R, Camand E, Chédotal A, Sotelo C, Dusart I: **Expression of Netrin-1, slit-1 and slit-3 but not of slit-2 after cerebellar and spinal cord lesions**. *Eur J Neurosci* 2005, **22**:2134-2144.

25. Barallobre MJ, Pascual M, Del Rio JA, Soriano E: **The Netrin family of guidance factors: emphasis on Netrin-1 signalling**. *Brain Res Brain Res Rev* 2005, **49**:22-47.

26. Burridge K, Wennerberg K: **Rho and Rac take center stage**. *Cell* 2004, **116**:167-179.

27. Mueller BK, Mack H, Teusch N: **Rho kinase, a promising drug target for neurological disorders**. *Nat Rev Drug Discov* 2005, **4**:387-398.

28. Mackay DJ, Nobes CD, Hall A: **The Rho's progress: a potential role during neuritogenesis for the Rho family of GTPases**. *Trends Neurosci* 1995, **18**:496-501.

29. Govek EE, Newey SE, Van Aelst L: **The role of the Rho GTPases in neuronal development**. *Genes Dev* 2005, **19**:1-49.

30. Huber AB, Kolodkin AL, Ginty DD, Cloutier JF: **Signaling at the growth cone: ligand–receptor complexes and the control of axon growth and guidance**. *Annu Rev Neurosci* 2003, **26**:509-563.

31. Mueller BK: **Growth cone guidance: first steps towards a deeper understanding**. *Annu Rev Neurosci* 1999, **22**:351-388.

32. Ren XR, Ming GL, Xie Y, Hong Y, Sun DM, Zhao ZQ, Feng Z, Wang Q, Shim S, Chen ZF *et al.*: **Focal adhesion kinase in Netrin-1 signaling**. *Nat Neurosci* 2004, **7**:1204-1212.

33. Liu G, Beggs H, Jurgensen C, Park HT, Tang H, Gorski J, Jones KR, Reichardt LF, Wu J, Rao Y: **Netrin requires focal adhesion kinase and Src family kinases for axon outgrowth and attraction**. *Nat Neurosci* 2004, **7**:1222-1232.

34. Chikumi H, Fukuhara S, Gutkind JS: **Regulation of G protein-linked guanine nucleotide exchange factors for Rho, PDZ-RhoGEF, and LARG by tyrosine phosphorylation: evidence of a role for focal adhesion kinase**. *J Biol Chem* 2002, **277**:12463-12473.

35. Swiercz JM, Kuner R, Behrens J, Offermanns S: **Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology**. *Neuron* 2002, **35**:51-63.

36. Samad TA, Srinivasan A, Karchewski LA, Jeong S-J, Campagna JA, Ji R-R, Fabrizio DA, Zhang Y, Lin HY, Bell E, Woolf CJ: **DRAGON: a member of the repulsive guidance molecule-related family of neuronal- and muscle-expressed membrane proteins is regulated by DRG11 and has neuronal adhesive properties**. *J Neurosci* 2004, **24**:2027-2036.

37. • Samad TA, Rebbapragada A, Bell E, Zhang Y, Sidis Y, Jeong SJ, Campagna JA, Perusini S, Fabrizio DA, Schneyer AL *et al.*: **DRAGON, a bone morphogenetic protein co-receptor**. *J Biol Chem* 2005, **280**:14122-14129.

Three papers [37•–39•] report that BMP2 and BMP4 bind to RGMa, RGMb and RGMc. These RGMs signal through BMP receptors (ALK3 and ALK6) and Smad1, and enhance signaling by BMP.

38. • Babitt JL, Zhang Y, Samad TA, Xia Y, Tang J, Campagna JA, Schneyer AL: **Woolff CJ, Lin HY: Repulsive guidance molecule (RGMa), a DRAGON homologue, is a bone morphogenetic protein co-receptor**. *J Biol Chem* 2005, **280**:29820-29827.

See annotation to [37•].

39. • Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, Samad TA, Campagna JA, Chung RT, Schneyer AL, Woolff CJ *et al.*: **Bone morphogenetic protein signalling by hemojuvelin regulates hepcidin expression**. *Nat Genet* 2006, **38**:531-539.

See annotation to [37•].

40. Lai M, Gluckman PD, Dragunow M, Hughes P: **Focal brain injury increases activin β mRNA expression in hippocampal neurons**. *NeuroReport* 1997, **8**:2691-2694.

41. Martinez G, Carnazza ML, Di Giacomo C, Sorrenti V, Vanella A: **Expression of bone morphogenetic protein-6 and transforming growth factor-β1 in the rat brain after a mild and reversible ischemic damage**. *Brain Res* 2001, **894**:1-11.

42. Setoguchi T, Nakashima K, Takizawa T, Yanagisawa M, Ochiai W, Okabe M, Yone K, Komiya S, Taga T: **Treatment of spinal cord injury by transplantation of fetal neural precursor cells engineered to express BMP inhibitor**. *Exp Neurol* 2004, **189**:33-44.

43. Hall AK, Miller RH: **Emerging roles for bone morphogenetic proteins in central nervous system glial biology**. *J Neurosci Res* 2004, **76**:1-8.

44. Foletta VC, Lim MA, Soosairajah J, Kelly AP, Stanley EG, Shannon M, He W, Das S, Massague J, Bernard O: **Direct signaling by the BMP type II receptor via the cytoskeletal regulator LIMK1**. *J Cell Biol* 2003, **162**:1089-1098.